NON-VOLATILE ORGANIC ACIDS IN SOME LIVERWORTS

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THE occurrence and metabolism of organic acids in liverworts had not been investigated until a chromatographic survey had been made by Allsopp[1]. However, he failed to detect any acids in eight genera investigated by him and was of the opinion that non-volatile organic acids may be totally absent in liverworts as compared with higher plants. Contrary to this finding, Das[2] reported the occurrence of aconitic acid (*trans* isomer) as a major constituent in *Riccia*, a genus not included in the survey made by Allsopp. The examination of *Riccia* had prompted further enquiry in this laboratory into other genera, not only because of the detection of acid itself but also owing to the unusual nature of accumulation of aconitic acid which is normally of restricted occurrence even in higher plants.

different acids based on a typical result with *Plagiochasma* are summarized in Table 1.

Thus a positive evidence is provided here for the occurrence of various non-volatile acids in the members of two different orders of Hepaticae examined, and it is likely that an investigation of other genera, including those investigated by Allsopp, would reveal a closely similar picture. The presence of *cis*-aconitic and malic acids both being Krebs-cycle intermediates makes it probable that their role in intermediary metabolism in liverworts may be similar to that in higher plants. However, aconitic acid itself is not easily detectable in higher plants except in a few cases such as sugar-cane, where it is found in appreciable quantities[5]. As for the appearance of aconitic acid mostly in the *trans* position, it was already argued[2] that

Table 1. Chromatographic Characteristics of the Acidic Constituents present in the Extract and their Identification

Acid	*R_F in *Sec.*-butanol/formic acid/H_2O (15 : 3 : 2; v/v)	*R_F in 1-Pentanol/5 M aqueous formic acid (1 : 1; v/v)	*p*-Dimethylamino-benzaldehyde†	Colour reaction to Ammoniacal $AgNO_3$‡	Colour reaction to Ammonium vanadate‡	*p*-Anisidine†	Identified as
1	0·879	0·654	Immediately red turns to wine red at 140°	White	Yellow	—	*Trans*-aconitic acid
2	0·719	0·273	,, — ,,	—	—	—	*Cis*-aconitic acid
3	0·545	0·345		Grey	Yellow	—	Malic acid
4	0·250	0·053	—	Yellowish red	Yellow turning grey-blue after 20 h	Pink	Mannuronic acid

* Unidirectional on Whatman No. 1 paper. Ascending direction in *sec.*-butanol solvent and descending direction in 1-pentanol solvent.
† Block, Durrum and Zweig (ref. 3).
‡ Buch *et al.* (ref. 4).

In the present investigation *Riccardia levieri* of anacrogynous Jungermanniales, *Plagiochasma* sp. and *Riccia billardieri* both belonging to the order Marchantiales formed the experimental material. These liverworts, available in the local hills, were collected, extracted in 80 per cent ethanol and the organic acids analysed by paper chromatography by means of the techniques described previously[2]. In each case the gametophytic vegetative thalli were used for the analysis.

A striking result of this investigation is the detection of *trans*-aconitic and malic acids in all the three genera. Also *cis*-aconitic acid, in traces, is now found to be invariably present along with its *trans* isomer in all cases, which was not evident in the earlier work. Another significant result is the identification, in addition to the acids mentioned, of mannuronic acid in *Riccardia* and *Plagiochasma*. Its presence in *Riccia* could not be confirmed at present owing to the lack of material, although preliminary evidence is in favour of it. All the acids, which were made visible on the developed chromatograms by spraying with *p*H indicators, were compared with the behaviour of authentic samples run alongside, by elution and subsequent co-chromatography and by selective chromogenic sprays. The results of identification of the instability of *cis*-isomer might have caused its transformation to the former.

The presence of mannuronic acid in liverworts is an interesting departure from angiosperms. In this feature liverworts appear to resemble algae since mannuronic acid is reported to occur among Phaeophyceae, where it is found as a constituent of alginic acid[7], a cell-wall polysaccharide. Phaeophyceae are marine organisms, and whether this comparative biochemistry between them and liverworts has any evolutionary significance should be considered. The polysaccharides of liverworts have not yet been studied and only further investigation will establish relationships, if any.

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INTERPRETATION OF DISTRIBUTIONS OF INDIVIDUAL RESPONSE TIMES IN MICROBIAL INFECTIONS

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A QUANTAL response, like death or formation of a local lesion, does not occur at the same time in different individuals inoculated with a pathogenic micro-organism. The mean response time is usually inversely proportional to log dose within a certain range, which implies that, in those hosts which respond, the organisms increase at a constant rate to reach a critical concentration at which the response occurs (Fig. 1*a*, refs. 1, 6).